A METHOD FOR STUDYING THE EXTERNAL ANATOMY OF COPEPODS

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The technique described below has been published in part (Gooding, 1960: 162), but we think that later modifications have proved sufficiently useful to justify this note. The method was developed for examining small copepods (under 2 mm in length), but can be adapted for larger ones. It seems suitable for any small crustacean.

We have found lactic acid to be the best clearing agent for the preparation of temporary mounts of whole or dissected copepods. Fresh, alcoholic, or formalin-fixed specimens become cleared within a few minutes to some hours, depending upon their size and the duration of preservation. When first placed in the undiluted acid, the copepods may become somewhat contracted, but soon regain, and thereafter retain, their normal size and shape.

In order to avoid rupture, obese forms or specimens with a thin cuticle are best transferred through mixtures of the medium in which they have been kept and lactic acid. Since the latter is dense, layering the fluids in a small dish is usually satisfactory: the original medium will evaporate slowly, leaving the specimens in the acid. Fluids with appreciable concentrations of dissolved salts, however, should be avoided, preferably by transferring the specimens first either to alcohol or to fresh-water.

Lactic acid renders the cuticle more supple than it is in most preservatives and thus more favorable for dissection, since setae, etc., are less easily broken off and lost. The refractive index is particularly suitable for study of fine detail. In our work, whole mounts or dissections of lactic acid-cleared material are prepared in the following manner.

In a wooden slide (of a soft, smooth-grained wood) measuring 75 × 25 mm (i.e., the dimensions of a standard microscope slide) and 1.5 mm in thickness, a centered hole 15 mm in diameter is bored. Using a metal stamp 22 mm in diameter, a depression is made on one surface of the slide (fig. 1a), forming a shelf 3.5 mm wide around the hole. A circular 22 mm cover glass of No. 1 thickness is held in place on this shelf by means of 2 minute drops of a suitable cement (for example, finger nail polish). One end of the slide may be buffed for labeling.

These slides can be prepared quickly in bulk by cutting a block of wood to the proper dimensions, boring a hole through the block, and then cutting strips of the correct thickness with a band saw. Such slides have several advantages over

the proprietary plastic holders suggested by Gooding (1960). They are more suitable optically, since their thinness permits correct adjustment of the microscope condenser for Köhler illumination; they can be made easily and cheaply; and they occupy less storage space.

With the slide upside-down, the specimen is placed in a small drop of lactic acid on the exposed surface of the cover glass. The animal may then be examined under the compound microscope by inverting the slide. The clarity imparted by lactic acid is often sufficient to make dissection unnecessary unless drawings of the appendages are to be made. For dissection we use minuten Nadeln, mounted in split sticks with sealing wax or in small pin-vises, with their tips sharpened, if desired, on a fine hone. A useful modification for the dissecting microscope is the device suggested by Harding (1939), which permits focusing by the movement of one knee, thus completely freeing both hands. Dissected parts of the animal should be pushed to the edge of the lactic acid drop. If examination under

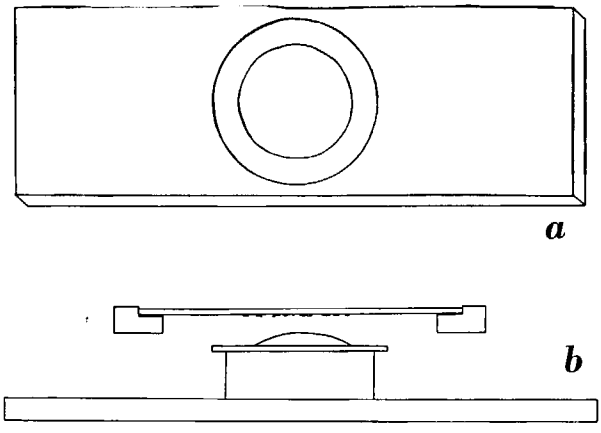

Fig. 1. a, view of upper surface of wooden slide; b, cross section of wooden slide and mounting pedestal.

the compound microscope shows that they are not in a suitable position, they can be reoriented under the dissecting microscope. Propping one part against another will often provide the desired angle of view.

One of the major advantages of this open-mount technique is that a single specimen can usually provide a full set of observations, since dissection can be stopped at any point and the results examined and/or drawn under the compound microscope, even with an oil immersion lens. In this way it is possible to produce a series of views (for instance, of the oral area with increasing numbers of appendages removed) which would be impossible by other methods, except by sacrificing several specimens. The constant alternation between dissecting and compound microscopes also helps to build up a unified picture of the interrelationships of parts within the whole animal. Another useful feature is that the copepods and their dissected parts suffer little or no compression, since they are hanging in a drop of fluid. Thus, more accurate views result.